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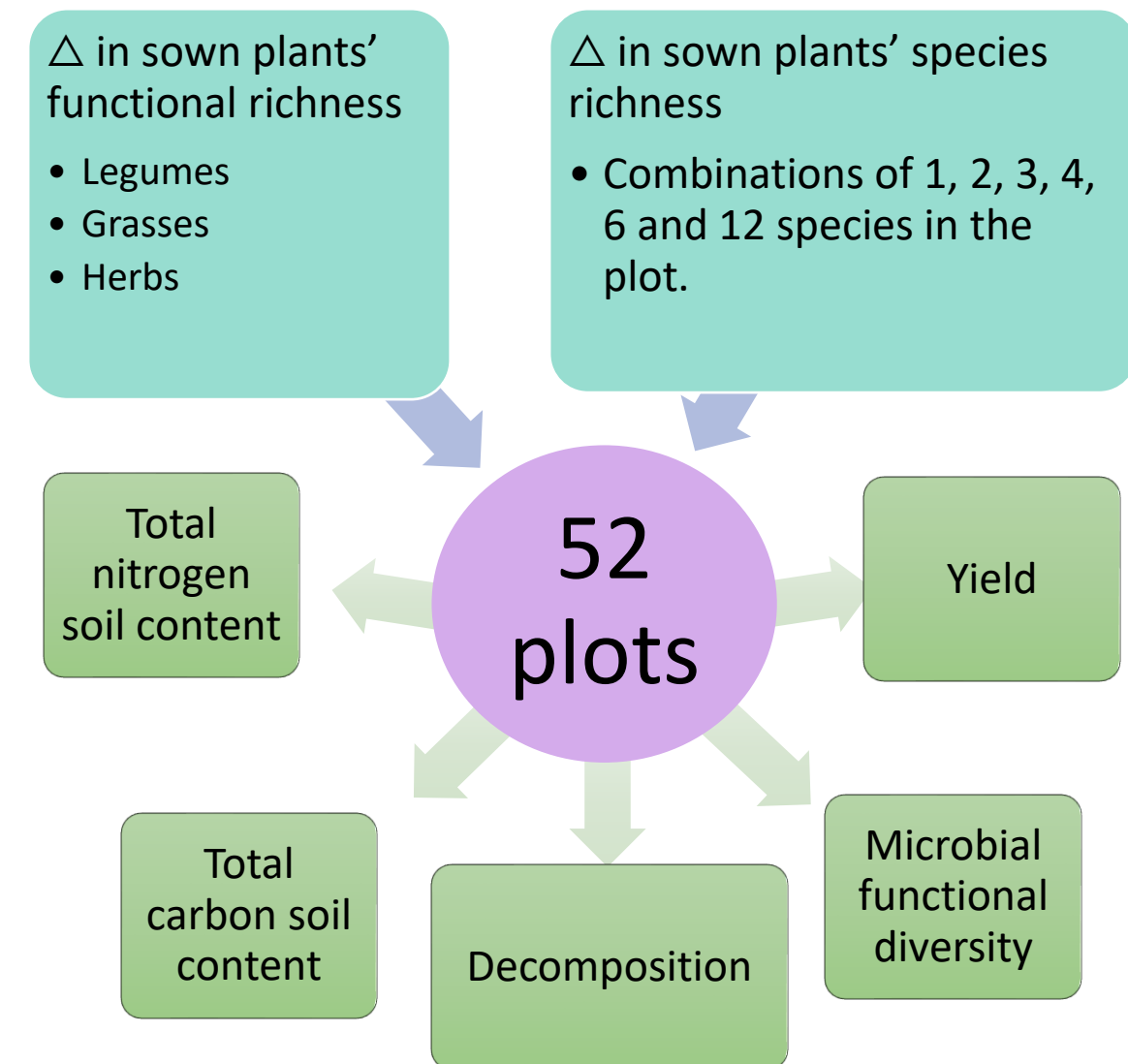
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INTRODUCTION

Crops represent the 26% of the Earth's Surface [1] and they have a key role in some ecosystemic services such as: the accumulation of organic carbon in the soil, the production of food for the cattle industry and the conservation of biodiversity [2]. However, bad policies in the management of these crops degrade the soils, contaminate and waste water and lower biodiversity [3]. To find solutions for these problems, the *LegacyNet* experiment project was created. This project consists in a network of experimental sites designed to investigate the beneficial effects of multispecies rotational crops and their legacy effects on follow-on crops in a rotation [4].

The field is divided in 52 plots of 3×7 m with different combinations of 1, 2, 3, 4, 6 and 12 species of 3 different functional groups: legumes, grasses and herbs. The main goal of the present work is to test the beneficial effects in the ecosystemic services of multispecies crops in contrast to monospecific crops using data collected in 2 years in the *Solsona* field on the following variables: microbial functional diversity of the microbial community, accumulation of total carbon and nitrogen in the soil, decomposition process and yield.



The data analysed in this work was collected in an experimental field of the *LegacyNet* experiment located in Solsona, Catalonia [5].

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MATERIALS AND METHODS

DATA ANALYSIS STRATEGY

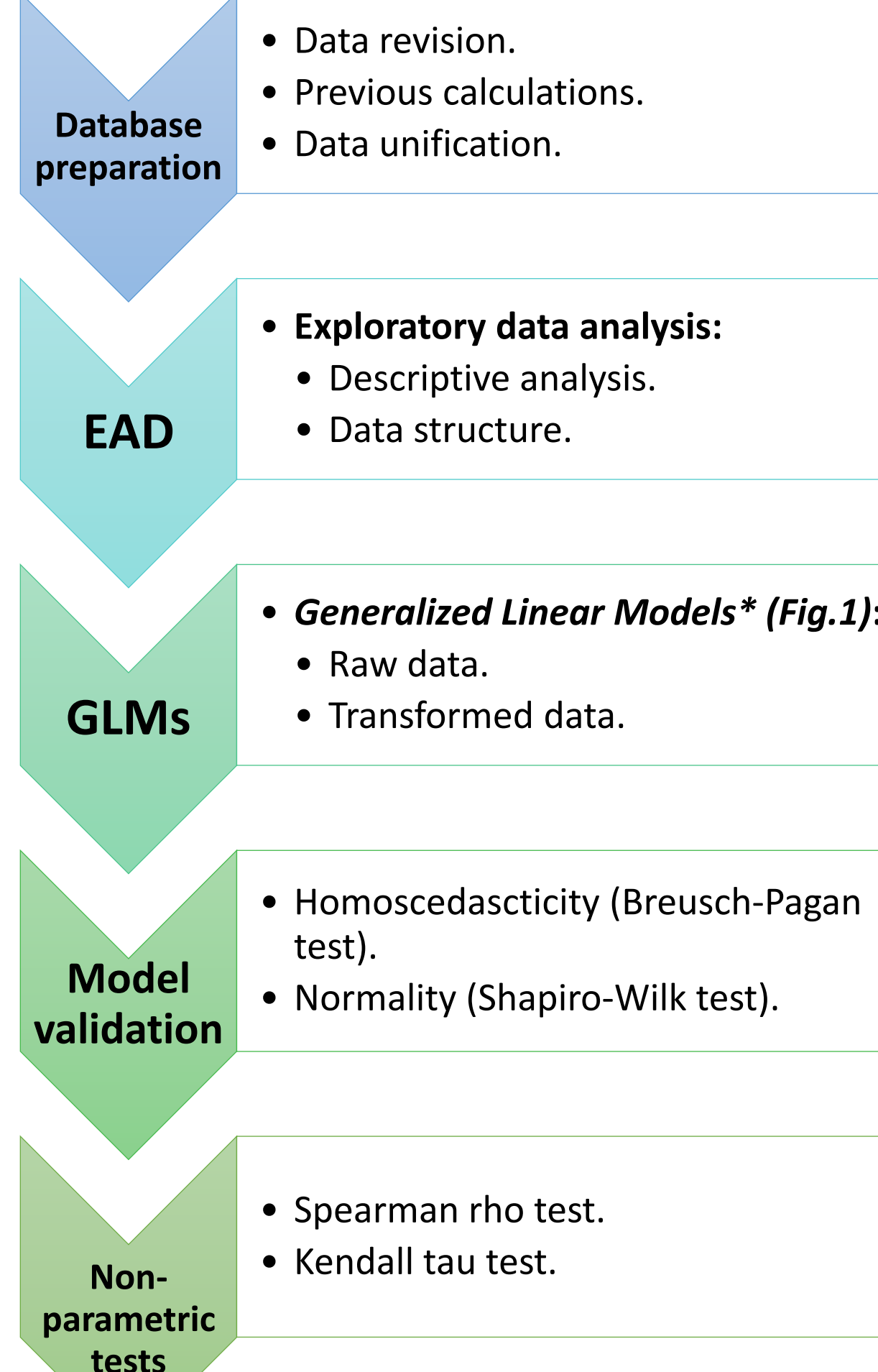


Table 1. Summarized information about the experimental methods used and EDA analysis (Units, Average and Sd (Standard deviation)) of the dependent variables with significant results analyzed in this study.

| Variables | Method | Units | Average | Sd |
|---|---|----------------------------------|---------|------|
| Yield | Harvest in the plot center 5 cm high and drying at 60 °C [5]. | $\frac{kg\ dry}{m^2}$ (Dry mass) | 0,33 | 0,17 |
| Relative mass of decomposed Rooibos tea | Teabag method [6]. | g | 0,27 | 0,06 |
| % of C _{Total} | Adapted Degtjareff method [7]. | % (Organic carbon) | 1,56 | 0,13 |
| % of N _{Total} | Micro-Kjeldahl method [8]. | % (Organic nitrogen) | 0,11 | 0,02 |
| Polymers | Microresp [9]. | % (de $\mu g\ CO_2 - C/g/h$) | 4,89 | 2,17 |
| MSIR | Microresp [9]. | $\mu g\ CO_2 - C/g/h$ | 53,56 | 5,80 |

*M1= glm (Dependent variable ~ ((Sown species/functional richness + Time) + (Interaction)))

Figure 1. General formula used for the GLM modelization.

RESULTS AND DISCUSSION

1. TOTAL NITROGEN (+) TIME

| Analysis type | Estimator | p-value |
|-------------------|-----------|----------|
| GLM with raw data | 0,039 | 3,32e-05 |

The increase of nitrogen (N) with time (Fig.2) contradicts the reported decrease in N with time due to plants nitrogen uptake in [10]. Possible causes for this results could be the conversion of inorganic N to organic form due to increased N immobilization, a high amount of root exudates accumulated in the soil with time, atmospheric nitrogen deposition or climatic conditions such as drought [11]. Nevertheless, the N increase is probably not related to an increase in sown functional richness (Fr) and to legumes nitrogen fixation [12], as no significant differences were found between Fr and N Total soil content.

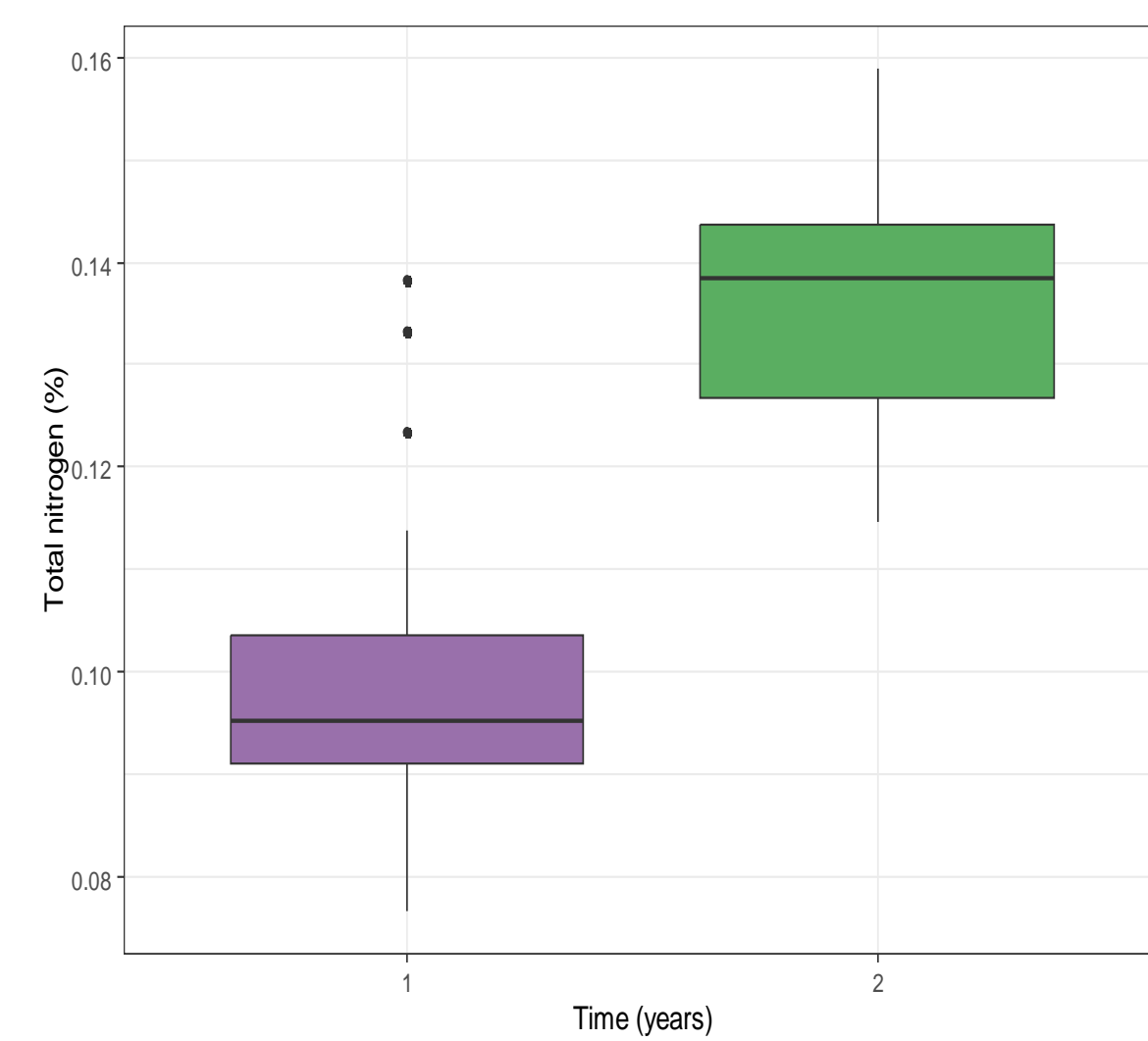


Figure 2. Graph of the Total nitrogen (%) variation for the different years.

2. MSIR (-) TIME

| Analysis type | Estimator | p-value |
|-------------------|-----------|----------|
| GLM with raw data | -9,68 | 4,01e-09 |

Variations of the Multiple Substrates Induced Respiration (MSIR) over time (Fig.3) are probably caused by changes in the physicochemical properties of the soil such as: low precipitation [13] and low temperature [14].

Microbial activity may also be affected by soil pH and total phosphorus content and to a lower extent by nitrogen and soil organic carbon content [15].

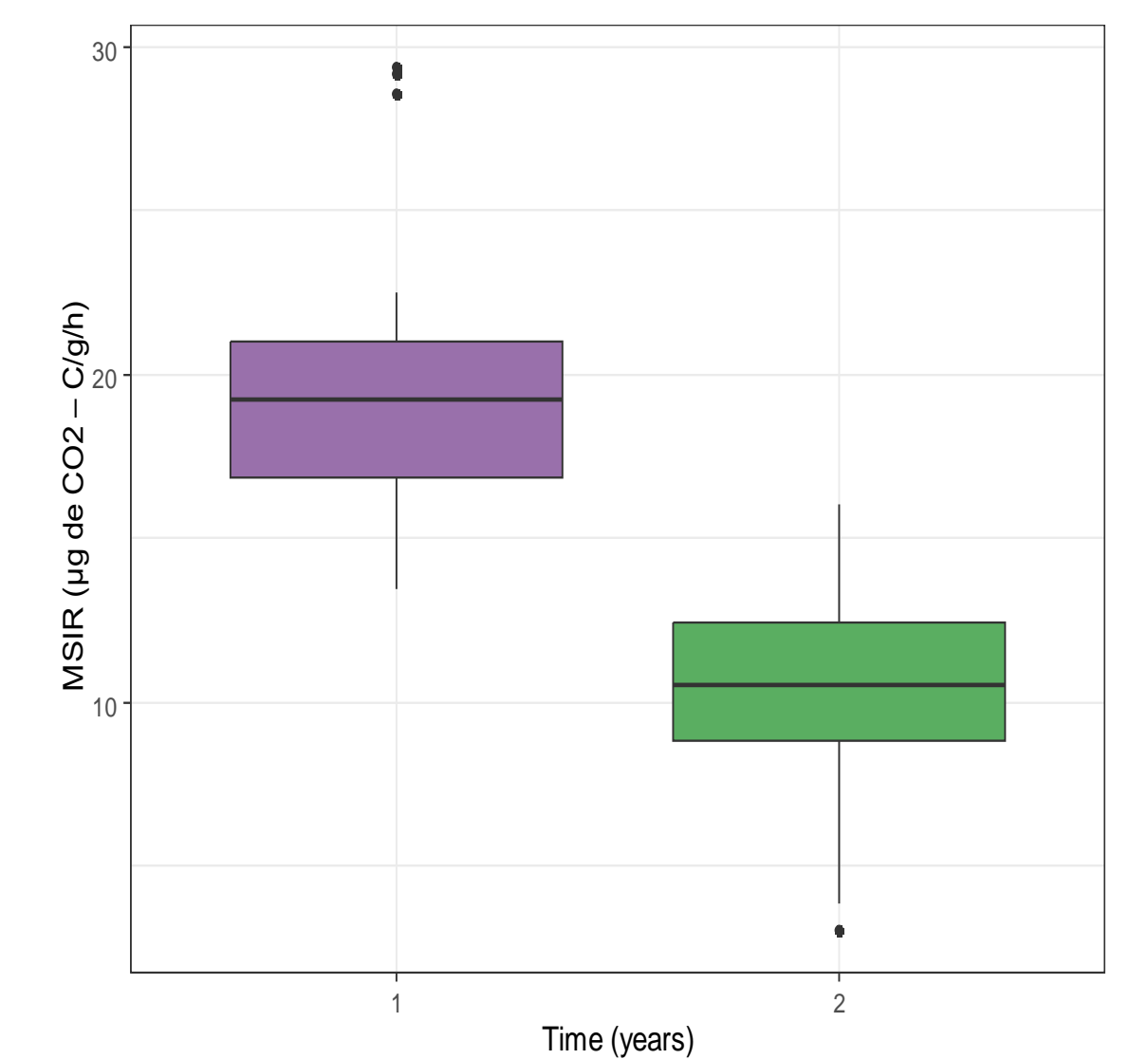


Figure 3. Graph of the MSIR activity (% of μg of $CO_2 - C/g/h$) variation for the different years.

3. MASS DECOMPOSITION OF ROOIBOS TEA (-) TIME

| Analysis type | Estimator | p-value |
|-------------------|-----------|----------|
| GLM with raw data | -0,0916 | 4,01e-09 |

The increase of the N soil content (Fig. 4) could have caused the decrease in the decomposition of more recalcitrant compounds, [16] such as Rooibos tea, which contains higher lignin content than green tea [6].

On the other hand, the decrease in MSIR may have also contributed to a lower recalcitrant compound decomposition.

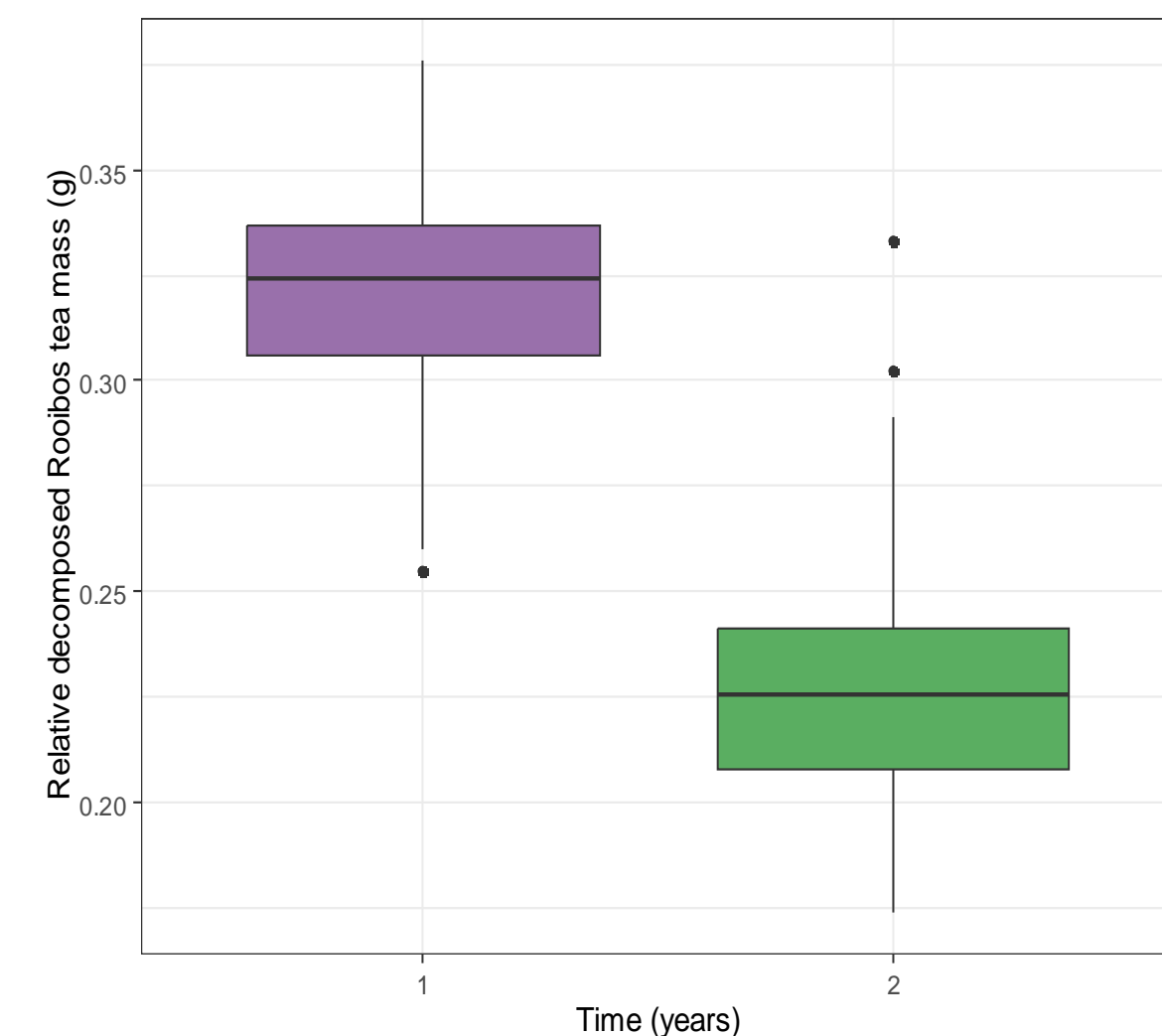


Figure 4. Graph of the Relative decomposed Rooibos tea mass variation (g) for the different years.

4. POLYMERS (+) TIME

| Analysis type | Estimator |
|----------------------|-----------|
| Non-parametric tests | Increase |

As can be seen in Fig.5, there is an increase in polymers (recalcitrant compounds) [17] consumption with time. This is contradictory to the fact that recalcitrant decomposition has decreased (lower Rooibos teabag decomposition) in this experiment.

More research is needed in order to understand the mechanisms of the relationship between the two variables.

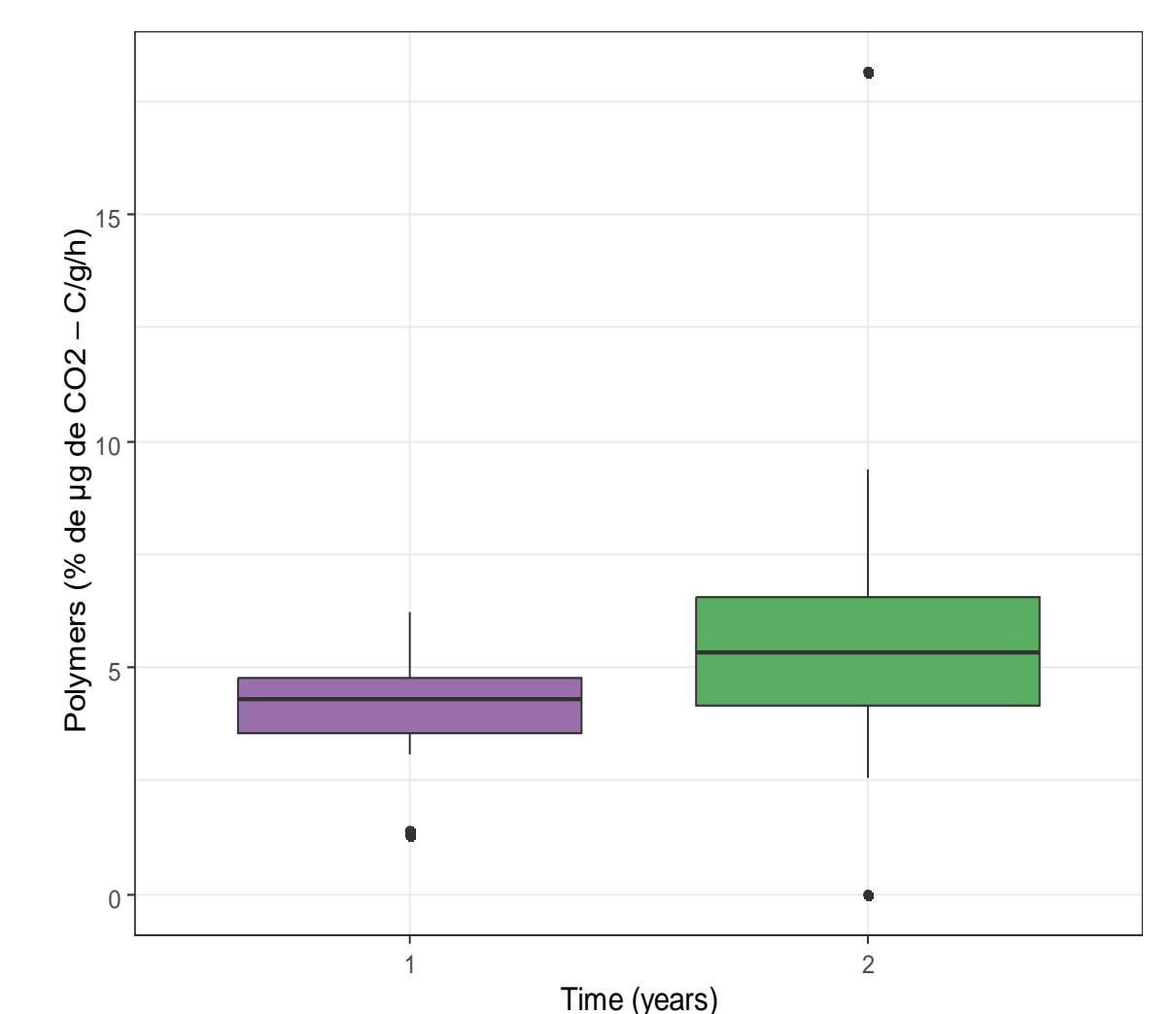


Figure 5. Graph of the microbial activity for the polymers substrate (% of μg of $CO_2 - C/g/h$) variation for the different years.

5. TOTAL CARBON (+) TIME (+) SOWN SPECIES RICHNESS

| Analysis type | Estimator |
|----------------------|--|
| Non-parametric tests | Increase (with Time and Sown species richness) |

Low MSIR (Fig. 3) might have also led to a higher persistence of exudates in the soil and therefore to an increase of C content with time (Fig. 6) [18].

In addition, the increase in the total C with higher sown species richness (Fig. 6) may be due to an increased root production and decreased root mortality, which may produce an increase in C stocks in high diverse plant communities [19].

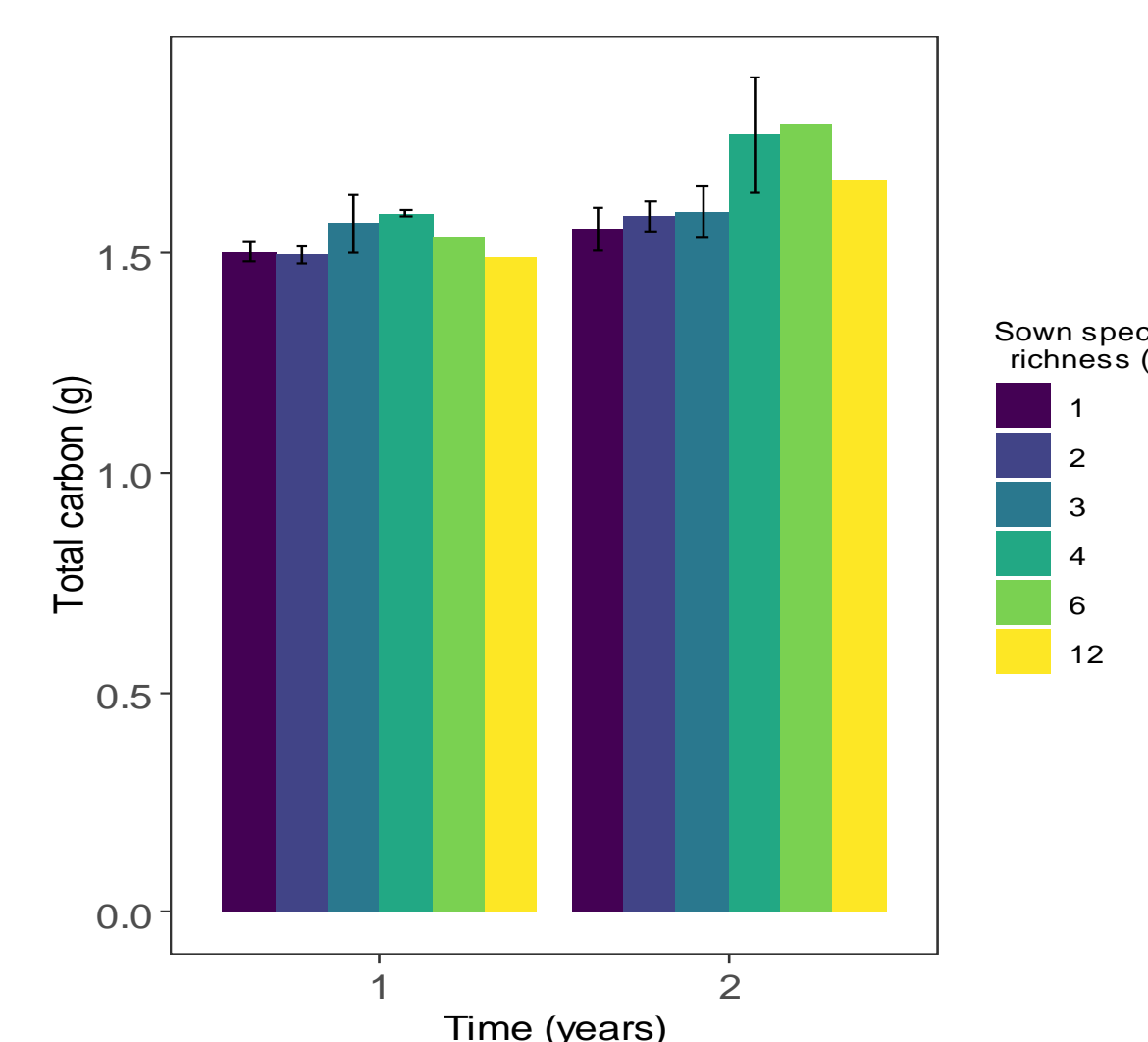


Figure 6. Graph of the Total carbon (%) variation for the different years and sown species richness.

6. YIELD (+) TIME (-) SOWN FUNCTIONAL GROUP RICHNESS

| Analysis type | Variable | Estimator | p-value |
|---------------------------------------|----------|-----------|---------|
| GLM with Log10 transformed yield data | Time | 0,19 | 0,008 |
| | Fr | -0,21 | 0,017 |

The increase of yield over time (Fig. 7) might be due to an increase of C total and N total content (Fig.2 and Fig.6) in the soil with time and might have had stimulated biomass yield [20]. Nevertheless, the decrease of yield with higher Fr contradicts previous results that found that increased plant diversity had a strong positive effect on biomass yield [21 and 22]. Even so, this trend is mainly seen in the first year and it has a weak significance value, therefore, more experiments should be performed to confirm this effect.

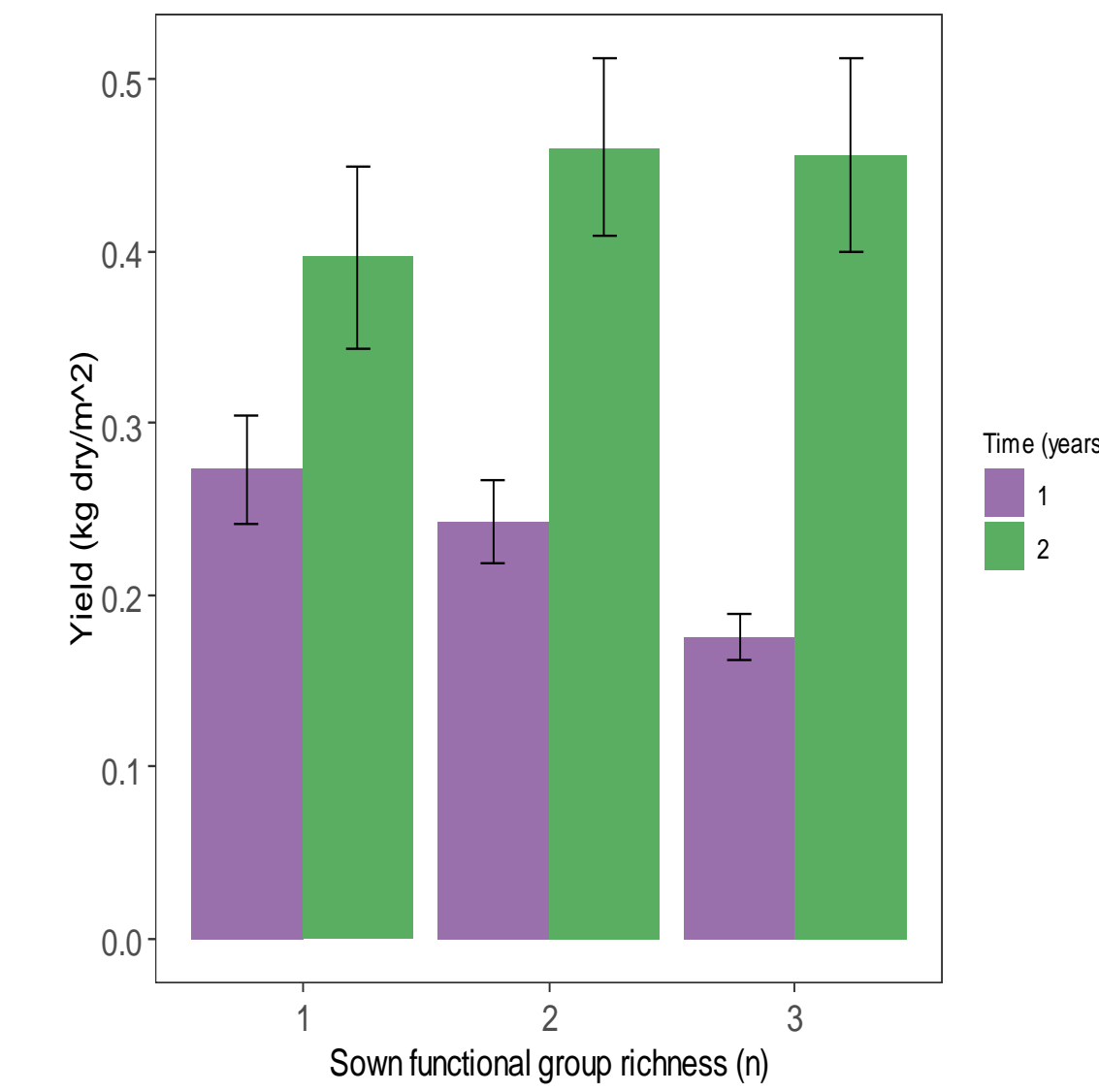


Figure 7. Graph of the yield variation for the different years and sown functional group richness.

CONCLUSIONS

- Most of the significant results were found between time and the different soil characteristics and may have been caused by changes in the weather conditions or variations in the soil composition by time or by the previous crop. It might be interesting to collect data of physical soil characteristics like pH, texture, and moisture content as well as weather parameters like temperature and precipitation to understand better these results.
- The short experimental time may explain lack of correlation between soil characteristics and sown species or functional group richness, as some soil characteristics may need more time to change[23].

- However, a significant effect of C Total with higher sown specific richness was detected. This may have been caused by increased exudate production by the roots and lower root mortality. This result is extremely important because the C cycle is slow and, still, C content presented differences in a period of 2 years.
- Due to the lower sampling size and the experimental design, the reliability of the results extracted in this study has been affected and varies depending on the statistical method used.

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